

³²P primer for 14/1 Vent
Human spleen DNA

ag N — ³²P 2633 (into the anchor primer)
follow P. 53 except use more ³²P ATP

~26th primer would ATP is 100% effective in labeling

Reagent	Concentration	Volume	Checkmarks
2633	159 μM	1 μl	✓
³² P γ ATP	6000 Ci/mmol	25 μl	✓
10 mCi/μl	10-21-94		✓
(1.67 μM ATP)			✓
5X Kinase buffer		625	✓
PNK 50 ^u /μl		0.25 μl	✓
		33.75	✓
(159 μM primer)			✓
(41.8 μM ATP)			✓
Any down 11C6 ladder			
10 μl H ₂ O			
1 μl ³² P dGTP			
15' 37°C			
1 μl EDTA			

37°C 30 min → 5' 55°C → add

spin col same as P154, 7, and 145, 3

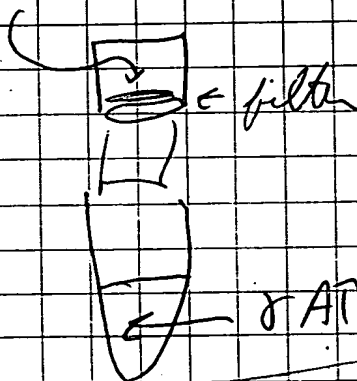
dilute ³²P 2633 with 100 μl H₂O (V_p = 133 now)

spin in microfuge in "micron 3"

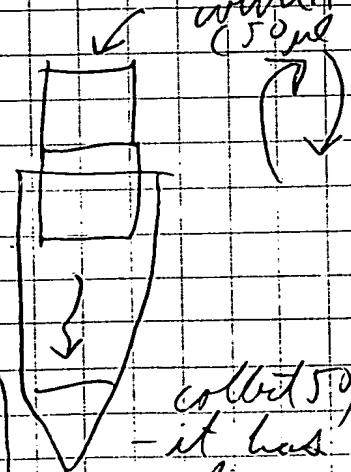
(amicon # 4240?) - after all went in, put

add 200 μl more H₂O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling back on micron 3. Maybe g force was too high on Beckman microfuge "E" model will skip separation of free ATP.

³²P 2633 is diluted only 33.75 fold for C_f = 4.71 μM

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Inspected & Understood by me,

Deena Pokrup

Date

10/24/94

Invented by

Recorded by

Date

10-19-94
10/24/94